IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
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Ming-Bo Wang et al.	)	Group Art Unit: 1631
	)	
Application No.: 10/780,638	)	Examiner: RUSSEL KALLIS
	)	
Filed: February 19, 2004	)	Confirmation No.: 2125
	)	
For: EFFICIENT GENE SILENCING IN PLANTS USING SHORT dsRNA SEQUENCES	)	

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Ming-Bo Wang, citizen of Australia, hereby state as follows:

1. I am a named inventor of the subject matter of the above captioned application.

2. I have read and understood the Office Action dated October 22, 2007. I understand the Examiner has rejected the claims as allegedly obvious over Wesley et al. The Plant Journal, 2001 , Vol 27 no. 6, pp. 581-590 in view of Yukawa et al. Plant Molecular Biology, 2002, Vol 50, pp 713-723.

3. I have also read and understood the Reply to the previous Office action dated January 25, 2007, wherein, in support of non-obviousness of the currently claimed invention, it was pointed out that the current application discloses for the first time that the use of PolIII type 3 promoter driven (short) hairpin RNA gene silencing is unexpectedly more effective than PolII (CaMV35S) driven hairpin RNA gene silencing for short double stranded RNA molecules (Reply page 8).

4. I understand from the Office Action dated October 22, 2007 that the Examiner has considered this argument but found it unpersuasive (Office Action at page 2).

5. I respectfully disagree with the Examiner. The specification in Examples 2, 3 and 5 disclosed results of experiments performed by me and/or under my supervision that demonstrated that for short hairpins (ranging from 21 to 94 basepairs, i.e. from 42 to 198

nucleotides), the PolIII type 3 promoter driven chimeric genes result in a more pronounced silencing of the expression of the target gene than similar PolII driven chimeric genes.

6. Example 2 disclosed a comparison of gene silencing efficacy for a 41bp hairpin RNA (comprising sense and antisense GUS sequences) transcribed from a construct driven by either a Pol II promoter (CaMV35S; pLMW53 see Table 2 on page 23, paragraph [0087]) or by a type 3 Pol III promoter (AtU3; pLMW58). These two constructs were introduced into two different tobacco plant lines each expressing a GUS gene as the target gene. The results of the MUG assay used to determine the expression level of the GUS gene in the transgenic plants containing both the target gene and hairpin construct (Table 3 page 24, paragraph [0091], indicated that the introduction of the Pol II driven chimeric construct (entries in the column under the heading "53") did not reduce the expression of the GUS gene (compare with entries in the column under the heading "untransformed"). Introduction of a similar chimeric construct under the control of a type 3 Pol III promoter did result in a significant reduction of the GUS expression (see lower MUG readings in the column under the heading "58").

7. Example 3 disclosed a comparison of gene silencing efficacy for a 41bp hairpin RNA (comprising sense and antisense GUS sequences) transcribed from a construct driven by either a Pol II promoter (CaMV35S; pLMW56 see Table 4 on page 25, paragraph [0094]) or by a type 3 Pol III promoter (AtU3; pLMW52). These two constructs were introduced into *Arabidopsis thaliana* plant lines expressing a GUS gene as the target gene. The results of the MUG assay, used to determine the expression level of the GUS gene, indicated that the introduction of the Pol II driven chimeric construct (Table 5 page 26, paragraph [0098], entries in the column under the heading "56") in this experiment did reduce the expression of the GUS gene (compare with entries in the column under the heading "untransformed"). However, introduction of a similar chimeric construct under the control of a type 3 Pol III promoter resulted in lower MUG readings, indicating a more efficient reduction of the GUS expression than for the Pol II construct (see lower MUG readings in the column under the heading "52").

8. In Example 5, Pol III promoters were tested for their efficiency in driving transcription of nucleotide sequences encoding short hairpin RNA molecules targeting endogenous genes, and their efficiency in silencing these target genes. The results are summarized in Table 6, page 28, paragraph [0104] for short hairpins targeting phytoene desaturase (PDS) gene expression, transcribed from either a chimeric gene under the control of

a Pol III promoter (U6 promoter) or a Pol II (CaMV35S) promoter. Introduction of the chimeric construct with the CaMV35S promoter resulted only in seedlings with either no bleaching or bleached cotyledons, but not bleached leaves, indicating only a weak silencing of the PDS gene by the Pol II construct, while most of the seedlings containing the chimeric construct with the Pol III U6 promoter were totally bleached, indicating stronger silencing of the PDS gene expression.

9. Attached (Exhibit 1) is a peer-reviewed scientific publication co-authored by me (Wang et al., 2008, RNA Vol 14, pp 903-913) which reports the data mentioned in the previous paragraph 8 and expands the analysis towards the further generation of progeny plants (T2 population). In particular, the difference in PDS silencing was even more clearly manifest in the progeny: 18 of 21 plant lines comprising the Pol III promoter driven chimeric gene showed intermediate to strong silencing, whereas only 5 of the 19 T2 plant lines comprising the Pol II promoter driven chimeric gene showed a visible but weak PDS silencing (Exhibit 1, page 904 right column, middle paragraph and Figure 3 (Exhibit 1, page 907).

10. Example 6 of the specification further describes the construction of chimeric genes encoding a short hairpin construct, having 42bp sense and antisense sequences separated by a 9bp spacer sequence, targeted towards reduction of expression of the ethylene insensitive gene EIN2 either under control a Pol III type 3 promoter (ATU3B; pLMW162 and pLMW163; Table 7, page 30, paragraph [0111]) or under control of a Pol II promoter (CaMV35S; pLMW157 and pLMW158; Table 7, page 30, paragraph [0111]). The Pol III promoter-driven construct gave significant EIN2 silencing as indicated by more vigorous leaf and root growth on ACC medium in comparison with wild-type *Arabidopsis* (Ler) or the Pol II promoter-driven plants. The corresponding Pol II promoter CaMV35S construct gave no significant EIN2 silencing in this experiment, showing that the Pol III promoter construct was much more effective than the Pol II promoter construct for EIN2 gene silencing. Exhibit 1, page 907, top of right column also describes that this endogenous gene, EIN2, was silenced more efficiently by the chimeric construct directed by the Pol III type 3 promoter than the CaMV 35S driven construct.

11. From the examples disclosed in the specification, a person of ordinary skill in the art would conclude that type 3 Pol III promoters are more effective than Pol II promoters such as the CaMV35S promoter when used to express relatively short hairpin sequences for gene silencing in plants, such as hairpin sequences ranging from about 19 basepairs to about 200 bp.

It is my belief that this discovery could not have been predicted from the state of the art at the time the invention was made, including the teaching of the references that have been cited by the Examiner.

12. It is therefore my opinion that the combined teachings of the cited references would not have allowed a skilled person to predict that type 3 Pol III promoters were more effective than the CaMV35S promoter for gene silencing when expressing relatively short hairpin sequences. At the time of the invention, a large variety of promoters of various types were known to a person of ordinary skill in the art. Those skilled in the art regarded the CaMV35S promoter as a strong constitutive promoter for directing expression of chimeric genes in plants, amongst the strongest known. Absent any specific evidence that another type of promoter would provide significantly better results than the CaMV35S promoter, there would have been no motivation for a person of ordinary skill in the art to use a different promoter and no predictability that other types of promoters would provide better results for silencing. Even more so, there would have been no reason for a person of ordinary skill to specifically select the type 3 polymerase III promoters that are recited in the claims of the present application over any of the many other types of promoters available for use instead of the CaMV35S promoter.

13. I hereby declare that all statements made herein of personal knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 24/11/08

Ming-Bo Wang